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ORIGINAL PAPER

Carbon nanotube-based electrochemical sensor for the determination of halobetasol propionate, a topical corticosteroid

Rajendra N. Goyal · Bharati Agrawal

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Abstract Electrocatalytic reduction of halobetasol propionate (HBP) at single-walled carbon nanotube (SWCNT) modified edge plane pyrolytic graphite electrode (EPPGE) was performed by square wave voltammetry and cyclic voltammerty in phosphate buffer of pH 7.2. The surface morphology of SWCNT/EPPGE was characterized by field emission scanning electron microscopy. The enhanced peak current (i_p) and lower reduction peak potential (E_p) at modified electrode as compared to bare electrode were obvious evidences for the electrocatalytic ability of SWCNT toward the reduction of HBP. The cathodic peak current varied linearly with concentration of HBP in the range 0.02 to 1 mM with sensitivity of 2.432 μA mM^{-1} and detection limit (3σ/slope) of 10 μM. The product of electrochemical reduction of HBP was characterized using FT-IR and ^{1}H-NMR spectroscopic techniques. A tentative mechanism for the formation of product was suggested and it was found that reduction of >C=O occurred at position three. The proposed methodology was successfully applied to the detection of HBP in pharmaceutical preparations.

Keywords Voltammetry · Halobetasol propionate · Carbon nanotubes · Pharmaceutical preparations

1 Introduction

Halobetasol propionate (6α, 11B, 16B)-21-chloro-6,9-difluoro-11-hydroxy-16-methyl-17-(1-oxopropoxy)pregna-1,4-diene-3,20-dione (**I**, HBP) is a topical corticosteroid which is widely used as anti-inflammatory, antipruritic, and vasoconstrictive agent [1, 2]. Topical corticosteroids are absorbed through the skin cells and prevent these cells from producing various inflammation-causing chemicals that are released when skin reacts to allergens and irritations [3, 4]. HBP is an ultra high-potency corticosteroid and is, therefore, suitable for the treatment of patients suffering with severe, localized corticosteroid-susceptible dermatoses such as plaque psoriasis, lichen simplex chronicus, and severe atopic dermatitis [5, 6]. Psoriasis is a chronic inflammatory skin disease of complex origin and two to three million people in the United State and approximately 1% of the world population is affected by this disease [7]. Since psoriatic plaques are thick, scaly, indurated, and dry, HBP has been found to be very effective in treatment of chronic, localized plaque psoriasis [8].

However, the use of HBP is associated with several side effects such as atrophy, postulation, leukoderma, acne, millaria, parasthesia, telangiectase, urticara, and striae [9]. Therefore, a number of studies have been published in literature describing analytical methods for determination of HBP. Reported methods for quantitation of this drug include spectrophotometric methods [1], polarographic method [10], high performance liquid chromatography, and high performance thin layer chromatography [11]. Most of the methods reported suffer from many disadvantages such as long separation time, requirement of expensive instruments, complicated procedure, and several derivatization steps are required prior to approach final analysis. Therefore, it is necessary to develop a simple, selective, sensitive, and inexpensive technique for the determination of compound I.

In recent years, voltammetric techniques have demonstrated the advantages of being both rapid and economical in the determination of several organic and inorganic compounds with high sensitivity and low detection capability. In

R. N. Goyal (✉) · B. Agrawal
Department of Chemistry, Indian Institute of Technology Roorkee, Roorkee 247667, India
e-mail: rngcyfcy@iitr.ernet.in

addition, electrochemical methods offer ease of operation, simple instrumentation, and are less sensitive to matrix effects than other analytical techniques [12, 13]. It has been found that the use of modified electrode enhances the sensitivity for electrochemical determination of biomolecules and drugs [14–16].

Searching the published methods for the determination of HBP shows that no electrochemical study has been performed. One of the reasons for this is the fact that the reduction of HBP occurs at high negative potential, which results in merging of the signal current with the background current. In all electrochemical experiments, the reaction of interest occurs at the surface of working electrode, therefore, the selection of working electrode can be a powerful tool for the success of any electrochemical reaction. Recently we have advocated the use of edge plane pyrolytic graphite electrode (EPPGE) for broad use in electroanalysis as it provides low background current, wide potential range in both positive and negative direction and improved electrocatalytical signals in comparison to other conventional electrode [17, 18]. Single-walled carbon nanotubes (SWCNT) have been utilized to improve electrocatalytic response of electrode and such intrinsic properties of SWCNT are believed to be favorable toward redox reaction of electrochemical species by shifting the peak potential in less positive or negative direction with enhancement of peak current simultaneously. Carbon nanotubes (CNTs) with their extraordinary mechanical and unique electrochemical properties have attracted much attention during last decade. Owing to huge surface area, subtle electronic characteristics, and strong adsorptive capability, CNTs have the ability to promote the electron transfer reactions of electroactive bimolecules and drugs [19–21]. A comparison of catalytic activity of SWCNT and MWCNT for the determination of amlodipine besylate and other biomolecules has been made [22, 23] and it is found that SWCNT exhibits higher activity as compared to MWCNT, hence, this article reports a convenient method for the assay of HBP, based on unique characteristic of SWCNT. At SWCNT-coated EPPGE the electrochemical response of HBP improves remarkably as reduction peak current increases and peak potential shifts to less negative potential as compared to bare EPPGE. After optimizing the experimental parameters, developed procedure has been used for the direct determination of HBP in pharmaceutical formulations. The product of reduction has been characterized by IR and NMR and the possible site of >C=O has been deduced.

2 Experimental

2.1 Instrumentation

All the voltammetric experiments were performed using Bioanalytical system (BAS, West Lafayette, USA) CV-50W voltammetric analyzer. The electrochemical cell used was a single compartment glass cell equipped with Ag/AgCl (3 M NaCl) as reference electrode (model BAS MF-2052 RB-5B), a platinum wire as an auxiliary electrode and SWCNT/EP-PGE as working electrode. The pyrolytic graphite plates and pieces were obtained from Pfizer Inc., New York, USA. Phosphate buffers [24] in the pH range (2.3–9.9) were prepared and the pH was measured using digital pH meter (model CP-901), Century India Ltd. Surface morphology of the bare and SWCNT/EPPGE was studied by using FE-SEM (JEOL JSM-7400) instrument.

Controlled potential electrolysis was carried out in a three-compartment cell equipped with three electrode system using pyrolytic graphite plate (area 6×1 cm^2) as working electrode, cylindrical platinum gauge as an auxiliary electrode and Ag/AgCl as reference electrode. UV–vis spectral studies were carried out using a Perkin-Elmer Lambda 35 UV–vis spectrophotometer. The FT-IR spectra were recorded by using a Perkin-Elmer 1600 series spectrophotometer using KBr pallets. GC–MS analysis was carried out with Perkin-Elmer Clares 500 Spectrometer in EI mode at 70 eV using HP-17 column. ^{1}H-NMR spectral studies were performed in an appropriate deuterated solvent ($CDCl_3$) with $SiMe_4$ as an internal standard using Advance 500 Digital NMR from Brucker. Chemical shift (δ) values have been indicated in parts per million (ppm).

2.2 Reagents and materials

Halobetasol propionate in powdered form was obtained as a gift from Symbiotic Pharma labs Ltd. Ankleshwar, India. SWCNT of purity >98% were purchased from Bucky, Houston, TX, USA. The SWCNT were analyzed for metal contents using atomic absorption spectroscopy and the Fe, Co, and Ni were found as 0.819, 0.412, and 0.207%, respectively. Phosphate buffer solutions of different pH and ionic strength were prepared according to the method of Christian and Purdy by mixing standard solutions of Na_2HPO4 and NaH_2PO4 [24]. HBP-containing creams and ointments of different companies were purchased from the local market of Roorkee. The medicinal samples (cream) of HBP obtained were Halovate (Glenmark pharmaceuticals Ltd., Mfg. Lic. No. MNB/05/182), Halox (Ranbaxy Labs. Ltd., Mfg. Lic. No. G/632), and Halobet-S (Ajanta Pharma Ltd., Mfg. Lic. No. KD-2590-A). All the chemicals used were of analytical grade and were purchased from Merck and double distilled water was used throughout the experiments.

2.3 Preparation of bare and SWCNT-modified EPPGE

A Pyrex glass tube of appropriate length and diameter was cleaned thoroughly and dried. One end of the glass tube is

filled with epoxy resin (Araldite, Ciba Geigy) up to a height of about 2 cm, with the help of a thin glass rod. The piece (2×3 mm^2) was then inserted in glass tube carefully from the other open end of the tube with the help of wire till 3/4th portion of it gets covered with epoxy resin to avoid any air pocketing between the tube and the graphite piece. The electrode was then allowed to stand for 24 h until resin solidified. The glass tube was rubbed on a sand paper till the graphite appeared at the resin end. Finally, the electrode was washed several times with distilled water in order to remove the fine powder adhered to the electrode surface of PGE. Mercury was filled into the glass tube and a copper wire was inserted to make proper contact of electrode to the outer circuit. The electrode surface was then cleaned by rubbing it on a sand paper, followed by washing with distilled water before using it for experimental purposes.

Modification of EPPGE was carried out by rubbing it on emery paper (P-600) followed by cleaning it with double distilled water. *N*,*N*-dimethyl formamide (DMF) was used for suspension of SWCNT and suspension was prepared by dispersing 0.5 mg of SWCNT in 1 mL of DMF by ultrasonic agitation. Amount of nanotubes casted on the electrode surface has a great significance toward the electrode response. Hence, different amount of nanotubes were casted in the range 5–40 μL at the electrode surface and electrode response was checked by recording voltammogram. Peak current was found to increase with increase in the volume of nanotubes casted up to 25 μL and then remained constant up to 40 μL. Therefore, 25 μL was selected as optimum amount of SWCNT for modification of electrode. This optimum amount of SWCNT suspension was coated on to the surface of bare EPPGE and was allowed to evaporate at room temperature. This modified electrode was used for further experiment. The surface morphology of bare and SWCNT/EPPGE was characterized by recording FE-SEM using Quanta 200 FE-SEM instrument. A comparison of FE-SEM images of the bare and SWCNT-modified EPPGE has been shown in Fig. 1.

2.4 Procedure

Halobetasol propionate is insoluble in water, hence, stock solution of HBP was prepared by dissolving the required amount of HBP in methanol. A known volume of stock solution of HBP was added to the 2 mL of buffer solution and the total volume was made to 4 mL with methanol. The solution was bubbled with nitrogen at a slow rate for 15–20 min before recording the curve. The BAS parameters were optimized and these optimized parameters were: initial (E): −400 mV, final (E): −1600 mV, square wave frequency (f): 15 Hz, sensitivity: 100 μA/V square wave amplitude (E_{sw}): 20 mV, step (E): 4 mV. All the potentials are reported with respect to Ag/AgCl reference electrode at an ambient temperature of 25 ± 2 °C.

2.5 Characterization of product

For characterization of product, about 10–12 mg of halobetasol was dissolved in 30-mL methanol and 30 mL of phosphate buffer solution of pH 7.2 ($\mu = 0.1$ M) was added. The solution was exhaustively electrolyzed by applying the potential ∼70 mV more negative than the reduction peak potential of HBP using potentiostat. To remove the interference of oxygen, nitrogen bubbling was done continuously at a slow rate. The progress of electrolysis was monitored by withdrawing a sample from bulk electrolytic compartment and simultaneously recording cyclic voltammograms and UV spectra at time intervals of 15 min. When the absorption peak in the spectra completely disappeared (∼after 24 h), the exhaustively electrolyzed solution was removed from the cell and lyophilized. The material obtained after lyophilization, was extracted with methanol and the colorless dried material obtained was used for further characterization.

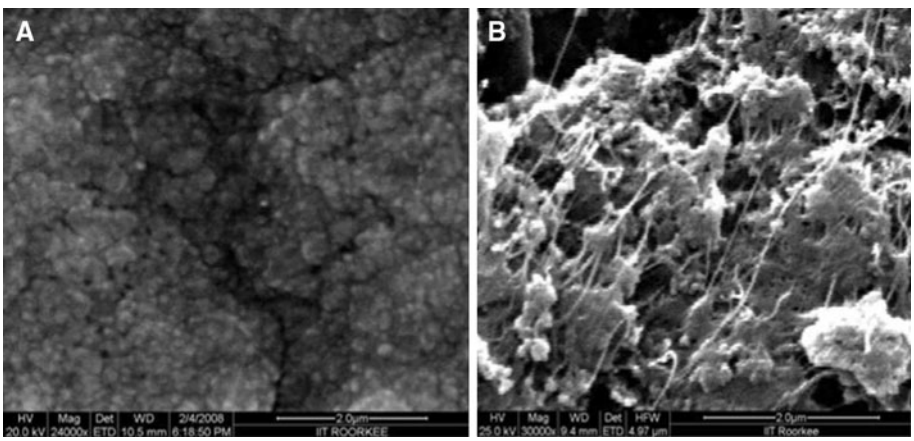


Fig. 1 Typical FE-SEM images of **a** bare EPPGE and **b** SWCNT/EPPGE

2.6 Analytical procedure

Halobetasol propionate is a trihalogenated agent and is available as 0.05% ointment and cream preparation. Each 1 g of halobetasol cream generally contains 0.5 mg/g of HBP. In order to detect HBP in cream and ointment, a known amount (5 g) of cream was weighed and dissolved in 25 mL of methanol and water in the ratio of 1:4 followed by heating it at water bath until cream melts. After allowing the residue to settle, the hot solution was filtered and the extract was used for further experiments.

3 Results and discussions

3.1 Cyclic voltammetry

Cyclic voltammetry is one of the widely used techniques providing the considerable information about the reversibility of the redox reaction. To accomplish this purpose, the electrochemical response of a solution of 0.5 mM of HBP was recorded by initiating the sweep in positive and negative directions. A well-defined reduction peak was noticed when the sweep was initiated in the negative direction. No other peak was noticed in the voltammogram. Thus, HBP is irreversibly reduced giving the single reduction peak at ~ -1295 mV at SWCNTs-modified EPPGE which is shifted to more negative potential (~ -1335 mV) with a mark decrease in peak current at the bare EPPGE. These results clearly reveal that modification of electrode by SWCNT catalyze the reduction of HBP occurring at the surface of working electrode. A typical cyclic voltammogram of HBP is presented in Fig. 2.

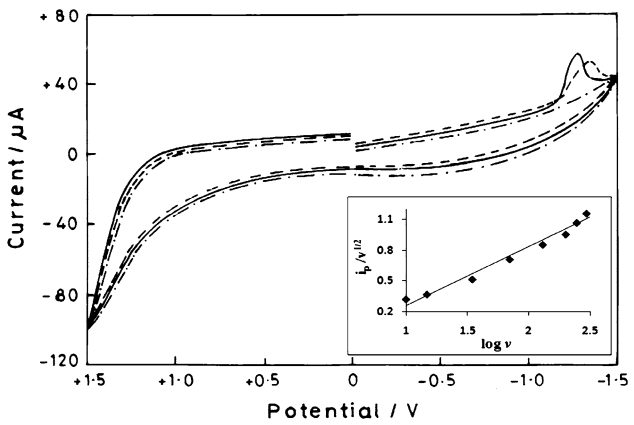


Fig. 2 Cyclic voltammograms obtained for blank PBS at SWCNT/EPPGE (*large dashed dotted line*) and 0.5 mM halobetasol (in 50% methanol) at pH 7.2 using bare EPPGE (*small dashed line*) and SWCNT/EPPGE (*straight line*) at 30 mV s^{-1}. Inset: variation of peak current (i_p) with scan rate

To establish the nature of electrode reaction, scan rate studies were performed in the range of 10–350 mV s^{-1}. Scan rate >350 mV s^{-1} could not be used as the peak changed to broad bump. Peak current due to the reduction of HBP was found to increase with scan rate and the dependence of peak current on scan rate can be expressed by following relations:

$$i_p/v^{1/2} = 0.576 \log v - 0.317$$

with a correlation coefficient of 0.97, where v is scan rate in mV s^{-1}. The linearity of plot of $i_p/v^{1/2}$ and log v as depicted in inset of Fig. 2 clearly indicated that the electrode reaction is controlled by adsorption phenomenon [25, 26].

3.2 Square wave voltammetry

Square wave voltammograms of 0.5 mM of HBP were recorded at bare and SWCNT/EPPGE. Figure 3 illustrates the voltammogram of HBP at bare and modified EPPGE at pH 7.2. Reduction of HBP occurs at lesser potential (~ -1265 mV) with enhancement of peak current at modified electrode in comparison to bare electrode. Appearance of reduction peak at less negative potential (~ -60 mV) with increament of peak current is an indication of catalytic behavior of SWCNT/EPPGE toward HBP reduction.

3.2.1 Effect of pH

Square wave voltammetric response of electrode is varied while changing the pH of supporting electrolytes. Effect of pH on reduction peak potential of HBP (0.5 mM) was studied in the pH range 2.3–9.9 by using the bare and

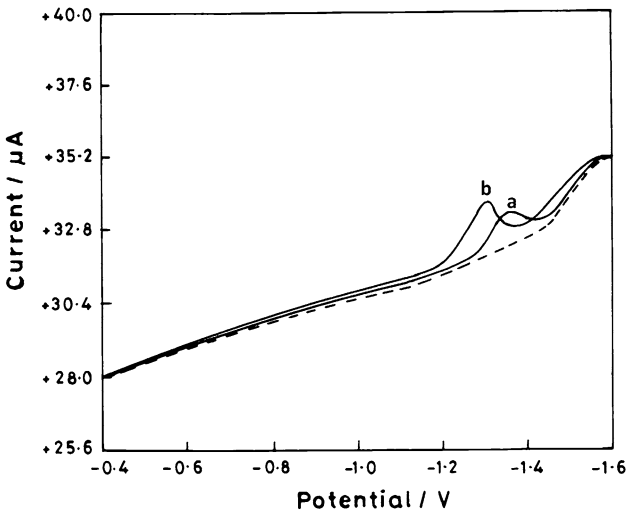


Fig. 3 Comparison of square wave voltammograms of blank PBS at SWCNT/EPPGE (*dashed line*) and 0.5-mM halobetasol (in 50% methanol) using (*a*) bare EPPGE and (*b*) SWCNT/EPPGE at pH 7.2

modified EPPGE. Reduction peak potential of HBP was found to be dependent and shifted to more negative potential with increase in pH as shown in Fig. 4a. The linear dependence of the peak potential of reduction peak on pH at bare and nanotubes-modified EPPGE can be represented by the relations:

$$-E_p/\text{ mV} = 884.7 + 56.30\ \text{pH} \quad \text{at bare EPPGE}$$
$$-E_p/\text{ mV} = 838.2 + 56.75\ \text{pH} \quad \text{at modified EPPGE}$$

having correlation coefficients of 0.996 and 0.995, respectively. The observed value of dEp/dpH suggested that equal number of electrons and protons are participating [27] in electrochemical reduction of HBP. As physiological pH is close to 7.2, the detailed studies were carried out at this pH.

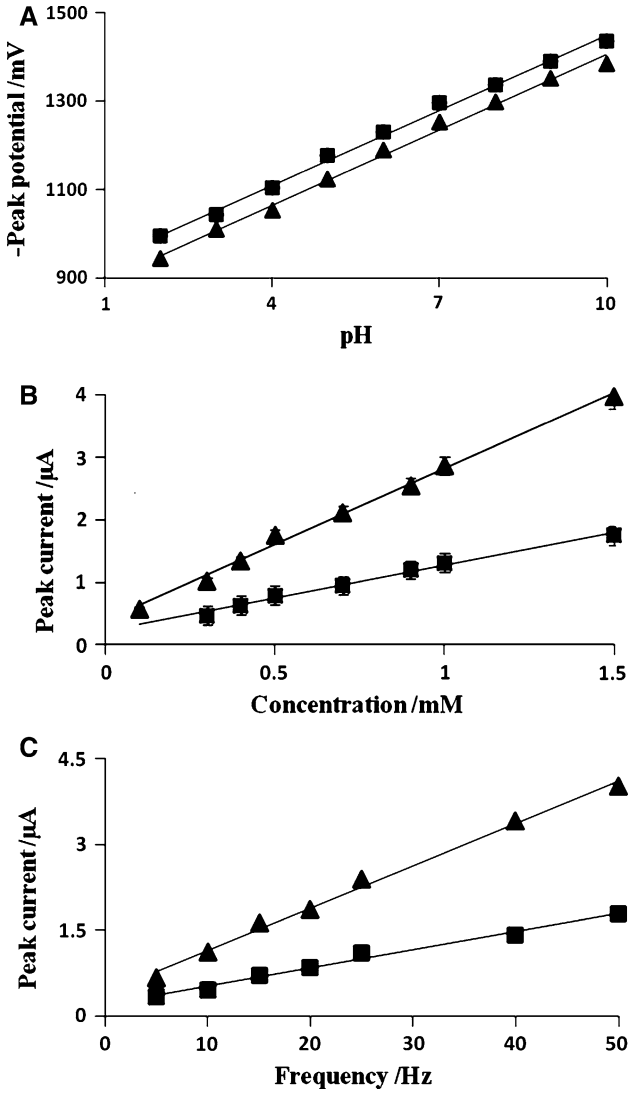


Fig. 4 **a** Effect of pH on E_p at bare EPPGE (*filled square*) and SWCNT/EPPGE (*filled triangle*), **b** Calibration plots at bare EPPGE (*filled square*) and SWCNT/EPPGE (*filled triangle*), and **c** Variation of peak current (i_p) with square wave frequency (f) at bare EPPGE (*filled square*) and SWCNT/EPPGE (*filled triangle*)

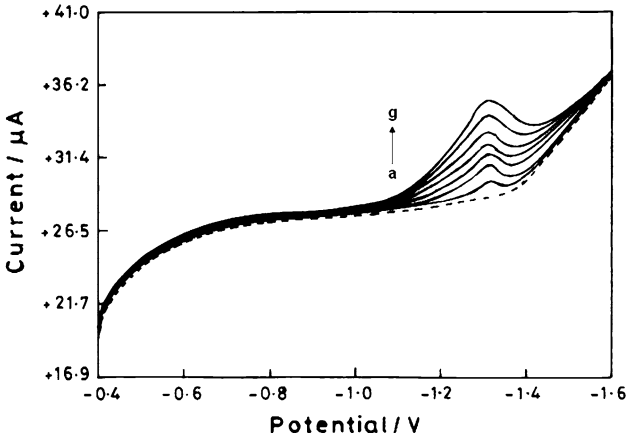


Fig. 5 Observed square wave voltammograms for (i) blank PBS (background) (*dashed line*) and (ii) increasing concentration of halobetasol (in 50% methanol). Curves were recorded at $a = 0.1$; $b = 0.3$; $c = 0.5$; $d = 0.7$; $e = 0.9$; $f = 1.0$, and $g = 1.5$ mM concentration using SWCNT/EPPGE in PBS of pH 7.2

3.2.2 *Effect of concentration*

The peak current of HBP increased with increasing concentration as shown in Fig. 5. The dependence of reduction peak current (i_p) on increasing concentration of HBP at bare and nanotubes-modified EPPGE is presented in Fig. 4b. Cathodic peak current of HBP increases linearly in the concentration range of 0.02 to 1 mM. The current values are obtained by subtracting the background current and are reported as an average of at least three replicate measurements. The dependence of reduction peak current on the concentration of HBP can be represented by equations:

$$i_p(\mu\text{A}) = 0.216 + 1.053\ \text{C} \quad \text{at bare EPPGE}$$
$$i_p(\mu\text{A}) = 0.384 + 2.432\ \text{C} \quad \text{at modified EPPGE}$$

having correlation coefficient of 0.990 and 0.994, respectively, where the term C represents millimolar (mM) concentration. The slope of the calibration plots corresponds to the sensitivity 1.053 and 2.432 μA mM^{-1} at bare and modified EPPGE, respectively. The limit of detection for bare and nanotubes-modified electrode were found to be 33 and 10 μM, respectively indicating the catalytic behavior of modified electrode toward the reduction of halobeatsol propionate.

3.2.3 *Effect of square wave frequency*

The effect of square wave frequency on peak current of HBP was studied in the frequency range of 5–50 Hz at pH 7.2. Studies at square wave frequency greater than 50 Hz could not be carried out because the reduction peak merged with the background. The peak current of 0.5 mM HBP

shows a linear increase with increase in square wave frequency (f) as shown in Fig. 4c, suggesting that the electrode process is adsorption controlled [28]. The linear relationship between peak current (i_p) and square wave frequency can be expressed by the relations:

$$i_p(\mu A) = 0.212 + 0.031 f(\mathrm{Hz}) \quad \text{at bare EPPGE}$$
$$i_p(\mu A) = 0.420 + 0.073 f(\mathrm{Hz}) \quad \text{at modified EPPGE}$$

with correlation coefficients of 0.987 and 0.994 for bare and modified EPPGE, respectively.

3.3 Analytical utility of proposed method in pharmaceutical preparations

Prior to examine the applicability of the proposed method for the determination of HBP, the method was applied to the analysis of drug in various samples of pharmaceutical formulations. Different cream and ointment were analyzed for HBP concentration at nanotubes-modified EPPGE in the phosphate buffer media of pH 7.2. By using the procedure mentioned as above, the extract of the filtered samples were further diluted by phosphate buffer solution of pH 7.2 so that the concentration of HBP lies in the range of calibration plot. Square wave voltammogram were then recorded and concentration of HBP was determined using calibration plot. The results obtained for HBP concentration are summarized in Table 1. The content for all assayed creams samples falls within the claimed amount, fulfilling the criteria of acceptance set according to the USP23 Uniformity of the Dosage Units [29]. Moreover, reference (labeled values) and observed amounts were compared. The calculated value obtained from student's t test is 0.21 for halobetasol cream samples (Table 1) at 95% confidence level indicates that there is no significant difference between the precision of claimed and observed amount.

The accuracy of the proposed method was evaluated by its recovery during spiked experiments (the addition of known amounts of pure drug to pre-analyzed formulations of halobetasol). For this purpose extract of filtered samples of HBP, creams and ointments were diluted two times with phosphate buffer. A typical square wave voltammogram of sample 1 at SWCNT-modified EPPGE is shown in Fig. 6.

Table 1 A comparison of observed and reported HBP concentration in pharmaceutical formulations using SWCNT/EPPGE

Cream sample	Reference amount (mg)	Observed amount (mg)[a]	Error (%)
Halovate™	0.199	0.195	−2.01
Halox™	0.199	0.200	0.50
Halobet-S	0.199	0.194	−2.51

[a] The R.S.D. value for the determination of halobetsol propionate in medicinal tablets was less than ± 3.1% for $n = 3$

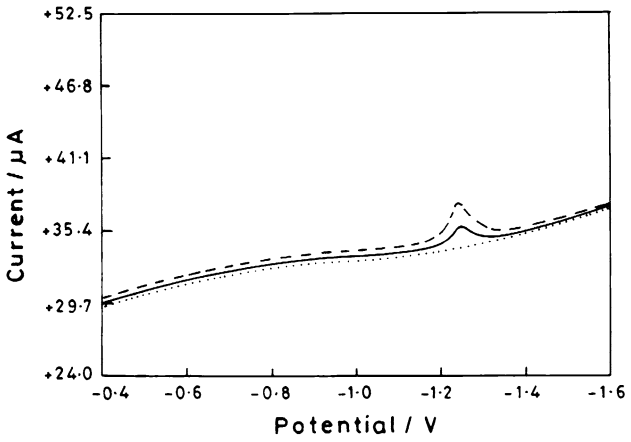


Fig. 6 Square wave voltammograms observed for blank PBS (background) (*dotted line*), halobetasol cream sample 1 (*straight line*), and sample 1 spiked with standard halobetasol (*dashed line*) using SWCNT/EPPGE at pH 7.2

A well-defined peak was noticed at peak potential of ~1267 mV due to the reduction of HBP. The sample was then spiked with known concentration of standard solution of HBP. From voltammogram of Fig. 6, it can be clearly seen that peak current of reduction peak having $Ep \sim -1267$ mV increases significantly with addition of HBP thereby confirming that it corresponds to the reduction of HBP. The concentration of HBP was determined using the regression equation keeping in consideration the dilution factor. The results obtained for different pharmaceutical samples, before and after the spiking, are tabulated in Table 2 and clearly indicate that the developed protocol can be easily used for the determination of HBP in pharmaceutical samples. In order to detect interactions of excipients viz, KCl, NaCl, and petroleum jelly as cream base, determination was carried out in their presence. As all these compounds are not reducible, they did not cause interference up to ~1,000 times concentration. The standard addition technique was used to the same preparations, which were analyzed by the calibration straight line. These

Table 2 Concentration of HBP observed in pharmaceutical samples at SWCNT/EPPGE using standard addition method

	Amount spiked (mM)	Amount detected (mM)[a]	Recovery (%)
Sample 1	0.0	0.42	–
	0.1	0.54	103.84
	0.5	0.91	98.92
	1.0	1.43	100.70
Sample 2	0.0	0.41	–
	0.1	0.50	98.04
	0.5	0.93	102.19
	1.0	1.43	101.41

[a] The R.S.D. for the determination was less than 2.4% for $n = 3$

results indicate the validity of developed method for the quantitative assay of halobetasol in commercial samples. The R.S.D, Bias, average recovery and R.S.D of recovery were found as <3.1, −1.34 (Table 1), 100.85, and 2.64% (Table 2), respectively. The mean percentage recovery showed no significant excipients interference, so the procedure was able to determine halobetasol in the presence of excipients and thus it can be considered specific with reliable analysis.

3.4 Stability and reproducibility of the modified electrode

Stability of SWCNT-modified electrodes for the determination of HBP was examined by measuring the current responses at fixed concentration of HBP at pH 7.2. The modified electrode was used daily and stored in air. The modified electrode showed a deviation in peak current of HBP by 3.34% after a single day, while after a week-modified electrode showed a relative standard deviation of 4.27%. This suggests that modified electrode possess sufficiently good stability.

The inter- and intra-day reproducibility of the proposed sensor was also evaluated. Deviation in current responses was calculated by using at least three replicate measurements of recorded voltammograms. Experimental results revealed that a R.S.D. of 0.76 and 1.24% was obtained while checking the intra-day and inter-day reproducibility, respectively, of the CNT-modified EPPGE. Only minimal decrease in current responses is attributed to the excellent stability of the modified electrode.

3.5 Product characterization

The UV spectral changes of HBP during electroreduction were recorded in the region 200–400 nm at pH 7.2. HBP exhibits a characteristic UV absorption at λ_{max} of 237 nm. With the progress of electrolysis the absorbance at λ_{max} decreased and no new absorption maximum was noticed as shown in Fig. 7. This indicates that the product obtained from electrochemical reduction of HBP does not absorb in the region 200–400 nm. The GC–MS of electrolyzed product of HBP exhibited a prominent peak at R_t ~11.20 min having molar mass of 488 (MH^+). As molar mass of HBP is 485; it increases by 2 amu after reduction and indicates that the reduction of HBP occur in a $2e^-$, $2H^+$ process.

Halobetasol propionate contains two spatially separated chromophores, i.e., cyclohexadienone moiety in ring A and carbonyl group at C_{20}. Therefore, reduction may occur at any or both carbonyl groups. To confirm the reduction site of HBP, FT-IR spectrum of HBP was recorded. The IR characteristic absorption bands were observed at 3439 (O–H str.), 2926, 2859 (C–H str.), 1718 (C=O str. in ester), 1664 (cyclic C=O str.), 1622 (acyclic C=O str.), 1450, 1385 (C–H def.), and 1076 cm^{-1} (C–O str.). The characteristic absorption near 1664 cm^{-1} due to cyclic C=O str. did not appear in FT-IR spectrum of the product, rather an extra absorption band near 3133 cm^{-1}(O–H str.) was observed. Thus, it is concluded that the site of reduction in HBP is at C-3 position.

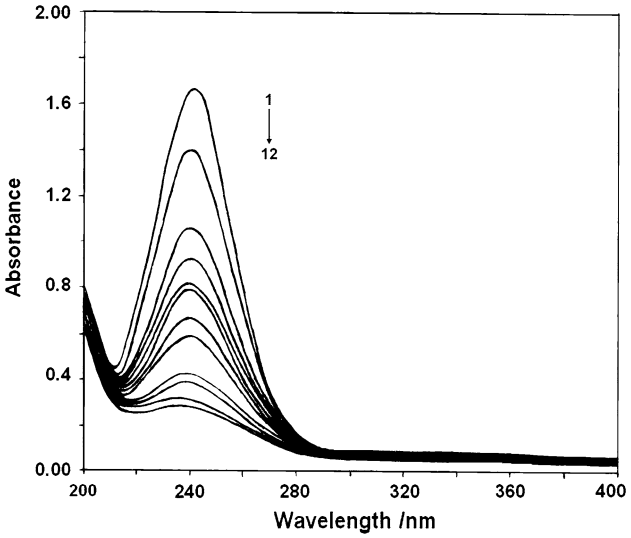
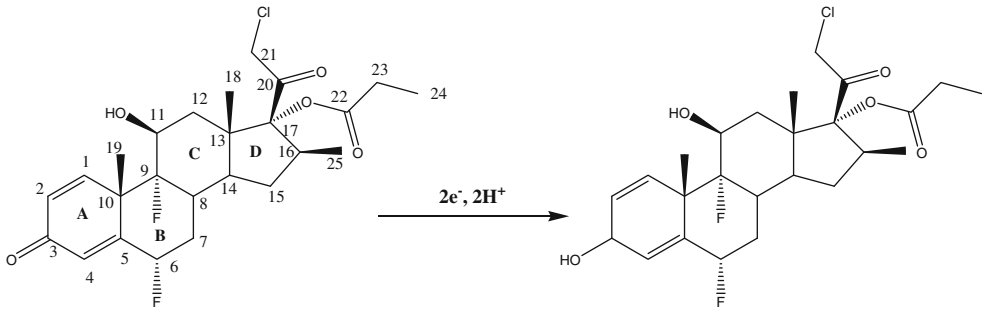


Fig. 7 Observed UV spectral changes during electroreduction of halobetasol at −1.3 V versus Ag/AgCl. The curves were recorded at (*1*) 0, (*2*) 15, (*3*) 30, (*4*) 60, (*5*) 120, (*6*) 180, (*7*) 300, (*8*) 480, (*9*) 600, (*10*) 900, (*11*) 1200, and (*12*) 1440 min of reduction

Table 3 A comparison of ^{1}H-NMR signals observed for halobetasol **(I)** and product **(II)**

No.[a]	I	II
1	7.15 (d, 1H)	6.43 (d, 1H)
2	6.34 (dd, 1H)	5.89
3	–	4.39 (bs)
4	6.42 (bs, 1H)	5.31
6	5.40 (ddd, 1H)	5.61 (ddd, 1H)
7	2.31, 1.65	2.26, 1.58
8	2.49	2.53
11	4.37	4.12
12	2.26, 1.43	2.34, 1.50
14	2.03	2.11
15	1.53, 1.20	1.51, 1.15
16	2.18 (t, 1H)	2.28 (t, 1H)
18	1.06 (s, 3H)	1.02 (s, 3H)
19	1.65 (s, 3H)	1.59 (s, 3H)
21	4.18	4.01
23	2.29 (q, 2H)	2.30 (q, 2H)
24	1.14 (t, 3H)	1.12 (t, 3H)
25	1.15 (d, 3H)	1.06 (d, 3H)

[a] See Scheme 1 for numbering



(I) (II)

Scheme 1 Tentative mechanism proposed for the reduction of HBP

To further confirm the site of reduction, ^{1}H-NMR spectra were also recorded. The NMR spectrum of HBP indicated the signals essentially similar to the ones reported in literature [11]. The signals corresponding to rings B,C, and D were found to remain unaffected while signals due to the ring A were strongly modified in the product. All chemical values of protons corresponding to rings B, C, and D were similar in the reactant and product, however, in the product an extra peak having chemical shift of 4.39 ppm (bs) was observed. This signal is assigned to the reduction of C-3 carbonyl group. Three of the olefenic –CH (ring A) were conserved but having lower δ value than observed in the reactant because >C=O was converted into CH–OH and deshielding effect of >C=O had been removed in the product. The details of all the NMR signals observed are shown in Table 3.

Above results clearly indicate that conjugated carbonyl group in ring A of HBP undergoes reduction and carbonyl group at position 20 remains unaffected during electrochemical reduction of HBP. It has also been reported earlier that conjugated carbonyl group undergoes easier reduction than the isolated one [30, 31]. Thus, the reduction in HBP occurs at C-3 position where keto group is converted to hydroxyl group by $2e^{-}$, $2H^{+}$ process as shown in Scheme 1.

4 Conclusions

The surface modification of EPPGE by single-walled CNTs improves its electrochemical properties by increasing the peak current and shifting the peak potential of HBP to the less negative values. The modified electrode offers several attractive advantages toward the voltammetric detection of HBP such as improved peak shape, high sensitivity, and low detection limit. Modified electrode also shows high stability and good reproducibility along with high accuracy which makes it appropriate for the determination HBP in clinical preparations. The origin of electrocatalytic properties of nanotubes has been assigned to the embedded metal impurities in CNT samples, edge plane-like defects which are present at the open ends of nanotubes and high surface area of CNT-modified electrodes [32, 33]. The purity of CNTs may affect the peak potential and peak current as observed earlier [33–35] and thus sample to sample change may cause some variation. However, such variations for CNTs for purity >98% will be minimal. The method eliminates the need for time consuming and tedious derivatization and extraction steps prior to analysis. Statistical calculations including student's t test, RSD%, Bias%, and recovery% clearly indicate that the analysis of medicinal samples using proposed method has excellent accuracy as the detected content was in good agreement with the labeled (reference) values. The characterization of the product indicates that the reduction takes place at carbonyl group present at position three and not at 20. It is necessary to mention that very few attempts [1, 36] have been made to determine HBP. Spectrophotometric determination using charge transfer complexes has reported a detection limit of 20 $\mu g\ mL^{-1}$, which is higher than observed in the present method. It is thus concluded that the proposed method is a good approach for sensitive determination of HBP owing to its simplicity, selectivity, specificity, and relatively short analysis time.

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